Clinical and Molecular Characteristics of Pediatric Gastrointestinal Stromal Tumors (GISTs)

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Background. To describe the clinical characteristics, molecular features, treatment, and outcome of six pediatric patients with gastrointestinal stromal tumors (GISTs). ***Patients and Methods.*** Retrospective clinical review of GISTs, seen at The Hospital for Sick Children (HSC) Toronto, over an 11-year period. All specimens were stained for the CD 117 and CD 34 antigens. Three specimens were sequenced for mutations in exons 9, 11, and 13 of the c-kit gene. ***Results.*** Five patients were evaluated and treated at HSC and one was referred for histopathological consultation only. The median patient age at diagnosis was 13.6 years, (6.9–14.8 years); four were female. All patients presented with anemia secondary to gastrointestinal (GI) bleeding. The disease was localized in five patients and two had other malignancies consistent with the diagnoses of Carney's triad. Immunohistochemical staining for CD 117 and CD 34 showed heavy cytoplasmic localization in all of the tumor cells. A novel point mutation of KIT in codon 456 of exon 9 was found in one case. Complete surgical resection was achieved in the five patients managed at our center and none received adjuvant therapies. Disease recurred locally in one patient. Four patients are alive and one is lost to follow-up. ***Conclusions.*** In children and adolescents, GISTs should be considered in the differential diagnosis of anemia secondary to GI hemorrhage. The absence of an exon 11 mutation and the identification of a novel mutation in exon 9 suggest that pediatric GISTs may respond differently to currently available targeted therapies and therefore should be studied within the context of collaborative group trials. Pediatr Blood Cancer © 2005 Wiley-Liss, Inc.

Key words: gastrointestinal; pediatric; stromal; tumors

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract in adults [1]. Previously diagnosed as GI smooth muscle tumors, GISTs are now defined as a distinct clinicopathologic entity, characterized by the expression of the KIT protein, which is detected by immunohistochemical assays for the CD 117 antigen [2]. *KIT* is a proto-oncogene encoding membrane bound receptor tyrosine kinase and its activation plays a central role in the pathogenesis of GISTs [3,4]. In most cases, the mechanism of oncogenic activation is a gain-of-function mutation, resulting in ligand-independent activation of the KIT receptor tyrosine kinase and an unopposed stimulus for cell growth [4,5]. The prevalence of *KIT* mutations in adult GISTs is as high as 90% [6]. These tumors are considered chemoresistant as less than 10% of malignant GISTs respond to chemotherapy [7,8]. Recently, it has been recognized that the administration of imatinib mesylate, a competitive inhibitor of *KIT* and platelet-derived growth factor receptor (PDGFR), produces a clinical response in over two-thirds of adult patients with this disease [9,10]. Although the clinicopathologic and molecular basis of GISTs have been well described in adults, there is a paucity of data in the pediatric population. Therefore, we investigated the clinical characteristics, molecular features, treatment, and outcome of six pediatric patients with GISTs seen at our center over an 11-year period.

PATIENTS AND METHODS

Review of the pathology archives at The Hospital for Sick Children, Toronto over an 11-year period identified 24 children with spindle cell neoplasms of the abdomen, excluding those of the soft tissues of the abdominal wall. Six of the tumors, diagnosed previously as leiomyomas, leiomyosarcomas, or GIST (Table I), were identified as GISTS based on the current histological and immunophenotypic criteria [2]. Five patients were treated at our center

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TABLE I. Patients and Tumor Characteristics

Patient	Age at diagnosis (year)	Sex	Year of diagnosis	Initial diagnosis	Location	Metastasis	CD 117	CD34	*KIT* mutation
1	14.0	Female	1994	Leiomyosarcoma	Stomach	No	+	+	Not done
2	13.3	Male	1994	Leiomyoma	Stomach	No	+	+	Exon 9
3	12.4	Female	1995	Leiomyoma	Stomach	No	+	+	Not done
4	14.8	Male	2000	Leiomyosarcoma (Carney's triad)	Stomach	Omentum	+	+	None
5	14.1	Female	1992	Leiomyosarcoma (Carney's triad)	Stomach	No	+	+	Not done
6	6.9	Female	2003	Gastrointestinal stromal tumor	Stomach	No	+	+	None

and one case was referred for histopathological diagnostic consultation only. After institutional review board approval, we obtained the following data from medical records: patient demographics, clinical presentation, treatment, and outcome. Direct sequencing for the presence of mutations in exons 9, 11, and 13 of the *KIT* gene was performed on three tumor specimens (case no. 2, 4, and 6) for which there was material available. Tumor tissues were digested in a proteinase-K buffer followed by DNA purification using a standard phenol/chloroform protocol. *KIT* coding sequences for exons 9, 11, and 13 were amplified by polymerase chain reaction (PCR) using primer pairs previously published [11]. The amplification products were size fractionated on an agarose gel, pretreated with Exonuclease I (USB) and Shrimp Alkaline Phosphatase (USB), and sequenced directly using forward and reverse primers. Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue sections using a specific antibody to *KIT*. The antibody used was rabbit polyclonal anti-human *KIT* (Dako, Carpinteria, CA) at a dilution of 1:20. All tissue sections were blocked for both endogenous peroxidase and biotin. The staining procedure was performed on the NEXES™ auto-immunostainer (Ventana Medical Systems, Tuscon, AZ). Immunodetection was carried out using the ABC system employing the Ventana DAB (3-3′-diaminobenzidine) detection system with Ventana signal amplification. The sections were counterstained with hematoxylin for nuclear detail. For negative controls, duplicate sections of each case and controls were stained, omitting the primary antibody.

RESULTS

The patient and tumor characteristics are depicted in Table I. The median age at diagnosis was 13.6 years, (range 6.9–14.8 years) and four were female. All of the patients referred to our institution (n = 5) initially presented to medical attention with symptoms of anemia. On further questioning, three had a history of hematemesis and two had previously been investigated after evidence of occult blood in their stool. One of the patients (case no. 6) was referred for investigation of iron deficiency anemia refractory to treatment, despite good compliance to therapy. On physical examination, the five patients had signs of an uncompensated anemia. One patient (case no. 4) was also found to have microcephaly, global developmental delay, and hypertension. None of the patients had symptoms of bowel obstruction.

Special investigations revealed an iron deficiency anemia secondary to an upper GI hemorrhage in all of the cases. An upper GI radio-contrast investigation was done in three of the patients and showed a filling defect in the stomach. A gastric tumor was confirmed in each of the patients by upper GI endoscopy (Fig. 1), abdominal ultrasound, and/or abdominal computerized tomography (CT). Evidence of metastatic disease was investigated by CT scans of the chest and abdomen. Four patients presented with localized disease and one had disseminated disease to the omentum (case no. 4).

Two of the children in this series were diagnosed with Carney's triad. (case no. 4 and 5). Case number 4 presented with the complete triad of gastric GIST, an extra-adrenal paraganglioma and pulmonary chondromas. The GIST

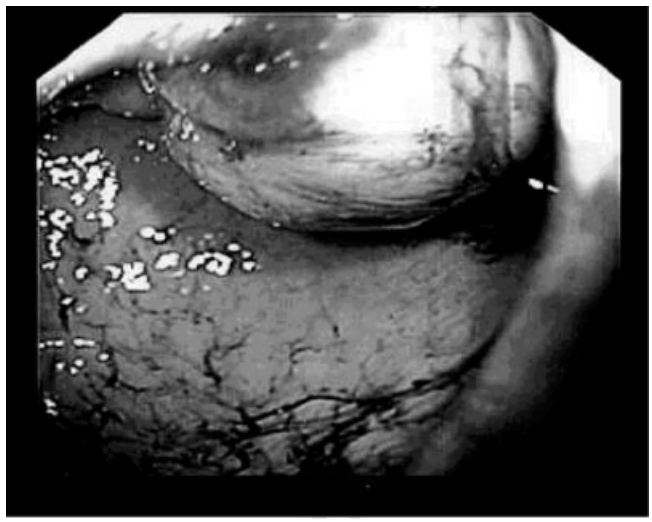

Fig. 1. Upper gastrointestinal endoscopy showing an exophytic mass in the gastric antrum.

had metastasized to the omentum and the primary gastric tumor had aggressive features characterized by a tumor volume of $5 \times 5 \times 2.5$ cm, multiple nodules, and areas of high mitotic rate. The gastric tumor and omental metastases were surgically resected and he received no adjuvant therapy. The patient is alive without evidence of a GIST. Case number 5 is a female, diagnosed with an incomplete triad at age 14 years when she presented with a localized gastric GIST and pulmonary chondromas. The tumor was surgically resected with clear resection margins. Four years later, she presented with a gastric recurrence and again the tumor was surgically resected. She is alive without evidence of a GIST.

Endoscopic biopsies in all patients were non-diagnostic and revealed normal gastric mucosa. Pathology specimens were obtained at the time of tumor resection in four patients and one patient had a CT guided biopsy prior to surgery. The initial pathological diagnosis of each tumor is summarized in Table I. Three of the tumors consisted of multiple nodules (case no. 4–6). Mucosal invasion was reported in two cases (case no. 5 and 6). Omental spread was evident in one patient (case no. 4) as a cystic, necrotic tumor nodule in the omentum. None of the tumors had metastasized to regional lymph nodes. By light microscopy, the pattern of the tumors varied from a spindled appearance to epithelioid, composed of moderately sized cells with abundant eosinophilic to clear cytoplasm. These lesions were focally hypercellular with abundant mitoses. Immunohistochemical staining for CD 117 and CD 34 showed heavy cytoplasmic localization in all of the tumor cells. (Fig. 2) Three specimens were available for molecular analysis (case no. 2, 4, 6). No sequence abnormalities in *KIT* exons 9, 11, and 13 were found in case no. 4 or 6. However, case no. 2 had a homozygous point mutation in codon 456 of exon 9 with a base substitution of $C > T$ resulting in an amino acid change from proline to serine (Fig. 3). This mutation was not present in normal tissue.

The tumors of the five patients seen at our institution were surgically resected. Complete resection with negative tumor margins was achieved in each case. None of the patients received adjuvant therapies. Disease recurred locally in one patient (case no. 5), 4 years after her initial presentation. Again the tumor was resected with no adjuvant chemotherapy administered. Four patients are alive and one patient is lost to follow-up.

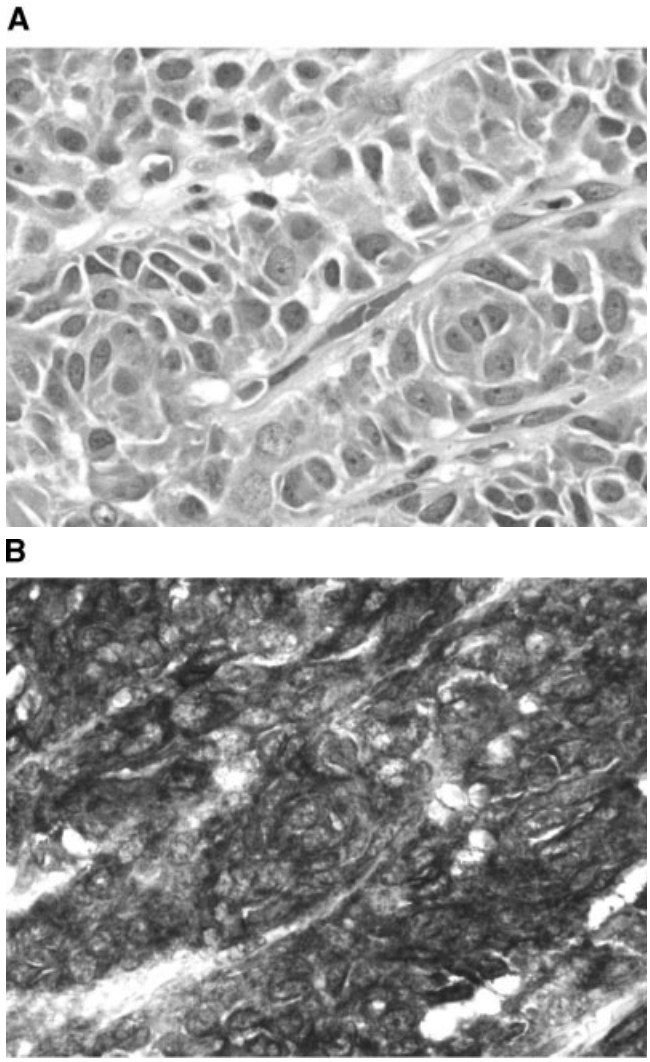

Fig. 2. **A**: Tumor cells with a rounded to polygonal configuration with abundant eosinophilic cytoplasm, supported by a delicate fibrovascular stroma. Hematoxylin and eosin staining. (Original magnification 1,480×.) **B**: Tumor cells demonstrating heavy staining for CD 117, as evident by the brown cytoplasmic deposits. (Original magnification 1,480×.)

DISCUSSION

To our knowledge, this is one of the largest reported case series of GISTs in a pediatric population from a single institution [12]. GISTs were previously diagnosed as GI smooth muscle tumors and only recently recognized as a completely separate histopathological entity. This is reflected in the date of diagnosis (pre 2000) in five of the six cases in this report, as three were originally diagnosed as leiomyosarcomas and two as leiomyomas. GISTs are defined as submucosal mesenchymal neoplasms of the GIT having spindle cell, epithelioid, or pleomorphic morphology and characteristic positive immunostaining for the CD 117 antigen [2]. On review of the pathology specimens, all were diffusely positive for CD 117 (*KIT*) defining them as GISTs. The most recent case (no. 6) was diagnosed in 2003 as a GIST.

This is also the first report of a *KIT* mutation in a GIST of a pediatric patient. This mutation is a novel homozygous point mutation in exon 9, located in the extracellular domain, resulting in an amino acid change at codon 456. Exon 9 mutations occur in up to 18% of adult GIST cases; the most common site reported in GISTs is

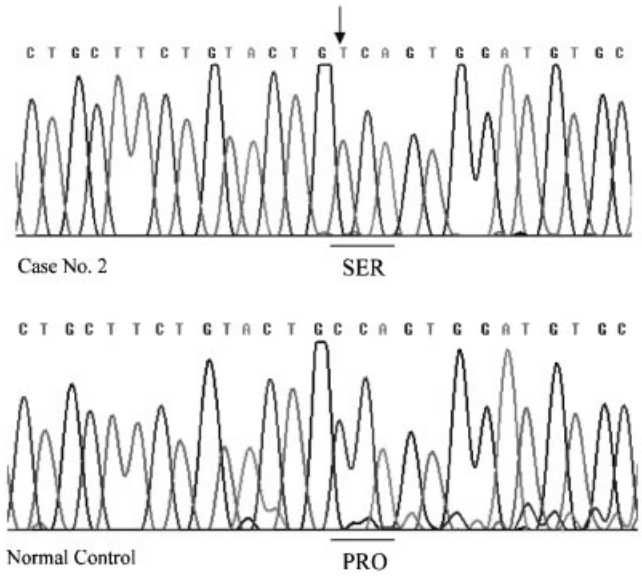

Fig. 3. Mutational analysis of c-kit exon 9 showing a point mutation in codon 456 with a base substitution of C > T resulting in an amino acid change from proline to serine in case no. 2.

an in-frame duplication of nucleotides resulting in the insertion of AY residues at codon 502 [11,13]. The result of this mutation is a ligand independent activation of the KIT receptor. Another mutation located nearby in exon 8 (residue 419), which is within the dimerization regulatory region, has been reported in acute myeloid leukemia [14]. Based on the analysis of these mutations, we hypothesize that the missense mutation seen in case no. 2 may also cause a functional change in the extracellular domain, perhaps by affecting dimerization, and this change may result in ligand independent activation of the KIT receptor. Further molecular studies will be required to determine the functional consequence of the mutation.

The findings of this limited series suggest that *KIT* mutations may be rare in children and may involve different exons than those seen in adults. Gain-of-function mutations in exons 9, 11, 13, or 17 of the *KIT* gene are present in up to 92% of adult GISTs, with exon 11 being the site of mutation in 70% of cases [6,11,14]. There are three pediatric cases reported in the English literature in which *KIT* mutations were sought and no sequence abnormalities were found in *KIT* exons 9, 11, or 13 [15,16]. Although we recognize the limitations of interpreting results from such a small cohort, these observations raise the intriguing possibility that pediatric GISTs may be biologically different from adult GISTs and therefore, their clinical response to targeted therapies such as imatinib mesylate may differ to that of adult GISTs. Imatinib mesylate, a competitive inhibitor of *KIT* and PDGFR α (PDGFRA), has been reported to induce a partial response of around 83% in adult patients whose tumors express an exon 11 mutant KIT protein, around 47% in those whose tumors express an exon 9 mutant isoform protein and no response in those with no detectable mutation of *KIT* or PDGFRA [17]. We did not examine any of the available specimens for mutations of PDGFRA. This may be important future objective as Heinrich et al. have shown a response to imatinib mesylate in a group of GISTs in adults lacking *KIT* mutations, but having PDGFRA mutations [17].

Predictors of unfavorable clinical behavior in adult GISTs include tumor size >5 cm, mucosal invasion, and a non-gastric primary site [18,19]. Four of the five patients investigated at our institution had GISTs >5 cm (cases no. 2, 4, 5, and 6) while mucosal invasion was reported in two cases (case no. 5 and 6). Mucosal ulceration is a common feature of gastric GISTs but has no predictive value or prognostic significance [19]. The common primary site was the stomach and this location of GISTs has been associated with a good prognosis [20]. The data on the prognostic value of *KIT* mutation are contradictory. Initial reports suggested that *KIT* mutations occur preferentially in biologically more aggressive GISTs [11,12,21]. More recently, Antonescu et al. reported that the presence of an exon 11 mutation may be associated with a less aggressive tumor behavior, while an exon 9 mutation may confer a more aggressive clinical behavior [21]. However, in our series, the patient with a *KIT* mutation in exon 9 had a tumor confined to the stomach and remains disease-free after resection of the primary tumor. Recently, Li et al. suggested that GISTs in children have features distinct from adults: female predominance, occurring in the second decade and a predilection for gastric location [16]. Aside from the female predominance, our findings support this statement as the median age of this cohort was 13.6 years (range 6.9–14.8 years) and the common primary tumor site was the stomach. All of our patients presented with iron deficiency anemia. This is of clinical significance, as GISTs should be considered in the differential diagnosis of children who present with anemia secondary to GI hemorrhage.

GI tumors are a component of Carney's triad, which is an association of gastric GIST, extra-adrenal paraganglioma, and pulmonary chondroma [22]. We were surprised to see that two of the cases had features compatible with this syndrome since this triad is rare, most often seen in young women and most patients present with two of the three tumors [22,23]. The pulmonary chondromas and extra-adrenal paragangliomas may only manifest later in adulthood and therefore the possibility of the triad should always be considered in children presenting with GISTs.

In conclusion, this case series of children with GISTs represents the largest case series reported and describes a novel exon 9 mutation of *KIT* not previously described in children. Furthermore, the prognostic factors described for GISTs occurring in adults may be unreliable in children and these patients might not be appropriate candidates for currently used targeted therapies. The

question as to whether GISTs occurring in the pediatric age group have a different biological behavior to those occurring in adulthood can only be answered within the context of a cooperative group trial that enrolls pediatric, adolescent and young adult patients. The Children's Oncology Group and the Southwest Oncology Group are currently undertaking such an initiative.

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